

Repeated dose of ketamine effect to the rat hippocampus tissue

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ABSTRACT

Aim: We aimed to determine the neurotoxic effect of repeated ketamine administration on brain tissue and if neurotoxic effect was present, whether this effect continued 16 days later using histological stereological method, a quantitative and objective method. **Materials and Methods:** Female rats were divided into three groups, each containing five rats. Rats in Group I were given 0.9% saline solution 4 times a day for 5 days. The rats in Groups II and III were given ketamine as intraperitoneal injections. Rats in Groups I and II were sacrificed on 5th day while the ones in Group III on 21st day. Cornu ammonis (CA) and gyrus dentatus (GD) regions in hippocampus tissue of rats were studied using optic fractionation method. **Findings:** There were significantly less number of cells in hippocampal CA and GD regions of rats from Groups II and III compared to the ones from Group I. Difference in cell number was also significantly higher in Group III than in Group II, but this difference was not as pronounced as the one between Groups III and I. **Conclusion:** Repeated ketamine doses caused neurotoxicity in rat hippocampus.

Key words: Ketamine, hippocampus, neurotoxicity, NMDA, optic fractionation

INTRODUCTION

Ketamine is one of the liquid analgesics commonly used in anesthesia for premedication, sedation, anesthetic induction and maintenance of general anesthesia. Due to tolerance development against opiates used as analgesics and in order to prevent hyperallergic reactions, use of low dose of ketamine has been suggested in recent years. Ketamine is commonly used in painful interventions such as burns and wound dressings because of its analgesic nature. It is preferred for sedative purposes (or for general anesthesia) in children since it suppress less the spontaneous breathing during magnetic resonance or computerized tomography screenings compared with other intravenous anesthetics.^[1]

Despite its beneficial effects, adverse effects of ketamine on patients during recovery such as hallucination and agitation limit its routine use.^[1] Some investigators consider ketamine neurotoxic,^[1,2] while others consider it as an anesthetic of the neuroprotective effect.^[3,4] Studies showed that single dose ketamine does not result in toxicity effect while repeated doses cause neurotoxic effect.^[1,2]

Hippocampus, considered to be the center of long-term memory in central nervous system, is a region of the brain sensitive to neurotoxic drugs.^[5,6] Hippocampus tissue of the brain is generally used to determine the neurotoxic effects of drugs in experimental studies.^[2,3] Hippocampus has two histologically different regions. Gyrus dentatus (GD) region has granular cells, whereas cornu ammonis (CA) region has pyramidal cells. Granular cells in GD region are the source of pyramidal cells in CA region, and resulting cells migrate toward CA region.^[7-9] The aim of the present study was to determine the neurotoxic effect of repeated ketamine administration on brain tissue and if neurotoxic effect was present on 5th day, whether this effect continued 16 days later using histological stereological method, a more quantitative and objective method.

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MATERIALS AND METHODS

This study was carried out after the approval by Local Ethic Committee in Gaziosmanpasa University, Experimental Research Center. Fifteen Wistar albino female rats, average weight 250-300 g, were used. The rats were randomly separated into three groups, each containing five rats.

Rats in Group I (control group) were given 0.2 ml 0.9% saline solution as intraperitoneal injections for 5 days, 4 times a day at 08.00, 12.00, 16.00 and 20.00 h using an insulin syringe. Rats in Groups II and III were given 0.2 ml 30 g/kg ketamine (Ketalar®, Pfizer, Turkey) solution as intraperitoneal injections for 5 days, 4 times a day at 08.00, 12.00, 16.00 and 20.00 h. Effect of anesthesia was observed for an hour following ketamine administration to animals.

Rats were maintained under optimum living conditions. They were fed the same kind of feed. Rats in Groups I and II were sacrificed using exsanguination method under ether anesthesia in an ether box on 5th day at 20.00 after the last ketamine application. Rats in Group III were sacrificed on 21st day, 16 days after the last dose.

After their death, rats were decapitated. Brain tissue was removed and placed in 10% formalin solution and embedded in paraffin for routine histological analyses. Five and 20 micron dissections were made from paraffin embedded tissues. Dissections were randomly sampled and stained with cresyle violet dye for histological examinations of hippocampus regions under a microscope. Total number of neurons was determined by counting the number of neural cells in the hippocampus region using optic fractionation.

Data obtained were evaluated using appropriate statistical methods (IBM SPSS Statistics 19, SPSS Inc., an IBM Co., Somers, NY, USA). Comparisons among the groups were performed using one-way analysis of variance. Two-way comparisons, on the other hand, were performed using Tukey's test, a *post-hoc* analysis. Kolmogorov-Smirnov test was applied to check whether the variables had a normal distribution.

RESULTS

Microscopic views of cells in CA and GD hippocampal regions of rats in study groups are given in Figure 1. Average numbers of neurons in hippocampal CA regions of study groups were compared. There were 62.33% less neurons in Group II than in Group I (control) ($P < 0.001$). Similarly, the average number of neurons in Group III was 34.25% less than the average of Group I ($P < 0.001$, Table 1 and Figure 2). When Groups III and II were compared, CA region of rats in Group III had 74.54% more neurons than those of the ones in Group II ($P < 0.01$).

In terms of neuron numbers in GD region of hippocampus of rats in different study groups [Figure 3], there was a 50.12% decrease in Group II compared to Group I. Neuron number was 33.88% less in Group III than in Group I ($P < 0.001$) [Table 1]. When cell numbers in CA regions of rats in Groups II and III were compared, Group III had significantly higher numbers (28.08%) than Group II ($P < 0.01$). Group III had 16.24% more cells in GD region compared to Group II ($P < 0.001$).

DISCUSSION

The present study showed that repeated ketamine doses resulted in decreases in neuron cells of rat hippocampus. The study employed optic fractionation method, one of

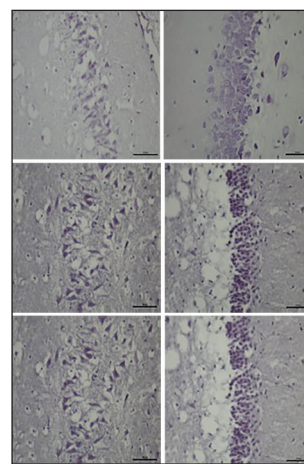


Figure 1: Microscopic view (x40) of cornu ammonis and gyrus dentatus regions of rat hippocampus

Table 1: Comparison of total number of neurons in CA and GD regions of hippocampus

Groups (n = 5)	Total number of neurons (1) (mean ± SE)	CV	Total number of neurons (2) (mean ± SE)	CV	P*
Group I	649864.4±21071.95	0.03	919195.40±82800.85	0.08	<0.001
Group II	244836.79±9388.26	0.03	458532.57±27649.69	0.05	<0.001
Group III	427350.00±26598.33	0.06	607762.80±25071.84	0.04	<0.001

*P value is valid for within and between groups in CA and GD regions. SE: Standard error; CV: Coefficient of variation; CA: Cornu ammonis; GD: Gyrus dentatus; 1: CA region; 2: GD region

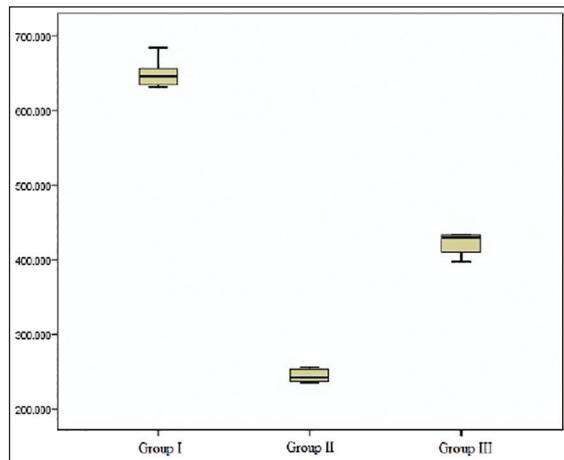


Figure 2: Distribution of neuron numbers in cornu ammonis region of rats from different groups

the most objective and accepted methods in the literature for cell counting.^[10,11] There is no study in literature using optic fractionation to determine total cell numbers in rat hippocampus.

In a study by Hayashi *et al.*,^[2] it was shown that fixed doses and intervals of ketamine administration to developing rats resulted in adverse effects on brain tissue. Compared to the control group, single dose ketamine did not increase neural degeneration. However, a 9 h 25 mg/kg repeated ketamine administration as 90-min intervals increased neural degeneration.

We administered repeated doses of ketamine to rats and found significant decreases in neuron numbers in CA and GD hippocampal regions of rats in treatment groups compared to the control group. In Group II, which had rats studied 16 days after ketamine application, the average number of cells was statistically lower than the control group but higher than Group II, which had rats studied right after ketamine application. This difference was thought to be caused by new cells produced during the 16 days from the last ketamine application to the time when rats were sacrificed.

The difference between Groups II and III for CA region was higher than the one for GD region. This difference reflected the cell production in GD region.^[7-9] In addition, when the effect of ketamine application was studied 16 days after the administration (Group III), cell number was higher than Group II. We hypothesize that this finding could be due to the migration of newly produced cells in GD region to CA region of the hippocampus.

There are some hypotheses about the mechanism by which ketamine causes neurotoxicity in brain tissue. In a study over monkeys,^[12] physiological and hemodynamic changes were observed. It was concluded that the cell deaths could

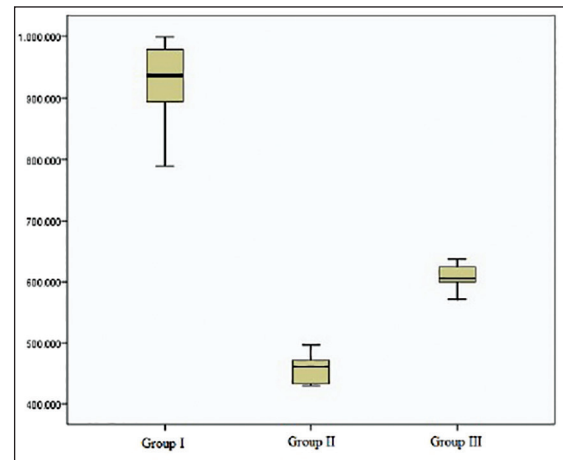


Figure 3: Distribution of neuron numbers in gyrus dentatus region of rats from different groups

be due to extended cerebral hypoperfusion following ischemia linked with lower arterial blood pressure and oxygen saturation.^[13]

Ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist. NMDA is a subtype of glutamate receptors associated with neural damage in central nervous system.^[14,15] Continuous stimulation of NMDA via ketamine may activate neural damage and apoptotic neural cell death.^[16-19] Ikonomidou *et al.*^[17] observed extended apoptotic degeneration in 7-day old developing rat brains treated with ketamine. Effect of repeated ketamine doses on hippocampus could be different in children, adults and elderly.^[4,18] In the present study, 8 weeks old rats were used, and significant decreases in cell numbers were detected in rats in Groups II and III.

The number of cells in the hippocampus was determined using stereological method. Number of cells was low both right after a 5-day ketamine application and 16 days after the application. However, in the hippocampus of rats studied 16 days after ketamine administration (Group III) there were significant improvements though not to the level in the control group.

CONCLUSION

Repeated doses of ketamine were shown to cause decreases in rat hippocampus cells in the present study. In addition, it was also found that cell numbers increased after the ketamine administration was stopped. The extent to which improvements can be made after repeated ketamine doses could be better illuminated through conducting further investigations using a higher number of rats, different ketamine doses and longer periods after ketamine administration.

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